CONTROLLED MAGNETOHYDRODYNAMIC FLUIDIC NETWORKS AND STIRRERS

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This invention was supported by funds from the U.S. Government (DARPA Grant No. N66001-97-1-8911 and DARPA Grant No. N66001-01-C-8056). The U.S. Government may therefore have certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATION

Applicant claims the benefit of provisional Application No. 60/409,359, filed September 9, 2002, which is incorporated herein in its entirety.

FIELD OF THE INVENTION

The invention relates to controlled, magnetohydrodynamically-driven, fluidic networks suitable for use in devices for processing and analyzing biological and chemical samples such as laboratories on chips and micro-total analysis systems. Placed within a temperature gradient, the fluidic networks of the present invention can further act as thermal cyclers, particularly of the type used for polymerase chain reactions (PCR). The invention also relates to magnetohydrodynamic stirrers that are capable of generating chaotic advection within a microfluidic conduit or chamber.

BACKGROUND OF THE INVENTION

In recent years, there has been a growing interest in developing minute chemical and biological laboratories, analytical devices, and reactors known collectively as laboratories on chips. The ability to perform chemical and biochemical reactions in such devices offers many benefits including reduced reactant and media volumes for safety and economy, and improved performance from increased thermal and mass transfer. In such devices, a spatially defined and controlled environment permits precise flow of reactants through the network. The flow of a fluid from one part of the device to another, and the efficient mixing of fluids are tasks that are far from trivial. In a micro-scale device such as a laboratory on a chip, mixing of fluids is a particular challenge as flows are at very low Reynolds numbers, turbulence is not available to promote mixing, and the insertion of moving components into these devices is difficult.

Electrostatic forces have been used to move liquids around such devices. These forces usually induce only very low flow rates, require the use of high electrical potentials, and can often cause significant heating of the solution which may be inappropriate for the materials being used or the reactions to be performed. The use of electromagnetic forces offers a means for manipulating at least slightly conductive liquids in microfluidic devices and systems.

The application of electromagnetic forces to pump and/or confine fluids is not new. It is known that magnetohydrodynamic (MHD) systems are capable of converting electromagnetic energy into mechanical work in fluid media. To date, MHD systems have mostly been used to pump highly conducting fluids such as liquid metals and ionized gases, to study ionospheric/astrophysical plasmas, and to control magnetic fusion devices. Recently, however, MHD micro-pumps in silicon and in ceramic substrates have been constructed demonstrating the

ability of such pumps to move liquids through microscale conduits. These efforts, however, have addressed individual pumping devices and have not provided an effective means for either the controlled movement of liquids through a microfluidic network or the efficient mixing of liquids in such microscale environments. Although it is envisioned that this invention will be mostly used in the context of minute devices, the concepts are not limited for small devices and can be applied for large devices as well.

Relevant publications, each of which are incorporated herein in their entirety, are identified as follows:

Bau, H. H., 2001, *A Case for Magnetohydrodynamics*, Proceedings of the 2001 ASME International Mechanical Engineering Congress and Exhibition, New York, NY 2001, November 11-16. CD. Vol 2.

Bau, H. H., Zhong, J., and Yi, M., 2001, *A Minute Magneto Hydro Dynamic (MHD) Mixer*, Sensors and Actuators B, 79/2-3, 205-213.

Bau, H., H., Zhu, J., Qian, S., and Xiang, Y., 2003, *A Magneto-Hydrodynamically Controlled Fluidic Network*, Sensors and Actuators B, 88, 205-216

Bau. H., H., Zhu, J., Qian, S., Xiang, Y., 2002, *A Magneto-Hydrodynamic Micro Fluidic Network*, IMECE 2002-33559, Proceedings of IMECE'02, 2002 ASME International Mechanical Engineering Congress & Exposition, New Orleans, Louisiana, November 17-22, 2002.

Jang, V., and Lee, S. S., 2000, *Theoretical and Experimental Study of MHD (Magneto-hydrodynamic) Micropump*, Sensors and Actuators A, 80, 84-89.

Lemoff, A. V., and Lee, A. P., 2000, *An AC Magnetohydrodynamic Micropump*, Sensors and Actuators B, 63, 178-185.

Lee, A. P. and Lemoff, A., V., *Micromachined Magnetohydrodynamic Actuators and Sensors*, U.S. Patent No. 6,146,103.

Qian, S., Zhu, J., and Bau, H. H., 2002, *A Stirrer for Magneto-Hydrodynamically Controlled Micro Fluidic Networks*, Physics of Fluids, 14 (10): 3584-3592.

Yi , M., Qian, S., and Bau, H. H. , *A Magneto-hydrodynamic (MHD) Chaotic Stirrer*, J. Fluid Mechanics, 468, 153-177.

Xiang, Y. and Bau, H. H., 2003, *Complex Magneto Hydrodynamic, Low Reynolds Number Flows*, Physical Review Letters E, 68, 016312-1 – 016312-11.

Zhong, J., Yi, M., and Bau, H. H., 2002, *A Magneto Hydrodynamic Pump Fabricated with Low Temperature Co-fired Ceramic Tapes*, Sensors and Actuators A: Physical, 96, 1, 59-66.

SUMMARY OF THE INVENTION

One aspect of the present invention is a controlled, magnetohydrodynamically-driven, fluidic network comprising a plurality of connected and individually controlled conduits each having at least one pair of opposing walls and at least one pair of electrodes disposed along the opposing walls, and at least one electrode controller in operational engagement with the electrodes for implementing an activation sequence comprising a current or potential across electrode pairs. In a preferred embodiment, the network further comprises an algorithm for determining the activation sequence. In operation, the fluidic network is provided with an at least slightly conductive fluid, is placed at least partially within a suitable magnetic field, and an electric field of a specific current or potential is applied for a predetermined period of time across electrode pairs and through the fluid within the network. In accordance with MHD principles,

the magnetic field is oriented approximately perpendicular both to the orientation of the electric field and to the axis of flow along the conduit. Interaction of the electric and magnetic fields generate volumetric forces, called Lorentz body forces that propel the fluid through the network. By the selective application of electric fields of specific currents or potentials at various points in the network for specific time intervals, the fluid may be directed with precision through the network in any desired pattern without the need for moving parts such as mechanical pumps or valves. In this manner, the control and flow of fluid through the network is similar to the control and flow of an electrical current in an electronic circuit.

The fluidic network is controlled by means of at least one electrode controller in operational engagement with the electrodes positioned on the sidewalls of the conduits. The controller or controllers implement an activation sequence of currents or potentials that are applied across the electrode pairs of the network. In one embodiment, the activation sequence is determined in accordance with an algorithm. The algorithm can be in any form that is capable of determining the magnitude, polarity and duration of current or potential across various electrode pairs throughout the network associated with generating a precise pattern of Lorentz forces for propelling the fluid in a controlled manner along any desired path through the network including, for example, an equation, a series of equations, a series of iterative steps, or software. The activation sequence may be entirely pre-determined by the algorithm or determined with the use of feedback generated by the operation of the network.

Another aspect of the invention is a controlled, magnetohydrodynamically-driven thermal cycler comprising the fluidic network of the present invention positioned at least partially within a temperature gradient. The imposition of a temperature gradient across the network allows fluid

to move through one or more zones of differing temperatures as it circulates within the network. By circulating materials through different temperature zones, chemical and biochemical reactions such as, for example, PCR may be readily accomplished. The thermal cycler described herein may be used singly or in combination, and may operate either as part of an MHD-driven fluidic network, as a component in a non-MHD fluidic system, or as a stand-alone device.

Yet another aspect of the invention is a MHD stirrer for use in microfluidic networks. The MHD stirrer comprises a conduit or cavity having at least two electrodes disposed therein such that complex secondary flows including flows characterized by chaotic advection are generated upon application of a current or potential across electrode pairs in a magnetic field. If two electrodes are used, at least one electrode must be movable between at least two positions to allow for the alternating application of current or potential across the electrodes in at least two different positions. If three or more electrodes are used, then the electrodes may be movable or fixed provided that a current or potential is alternately applied between electrode pairs in at least two different positions. In either embodiment, the application of a current or potential across the electrodes induces at least two different alternating flow patterns which in turn induces chaotic advection. The MHD stirrer may be used singly or in combination, and may operate either as part of an MHD-driven microfluidic network, as a component in a non-MHD microfluidic system, or as a stand-alone device.

A further aspect of the invention is a method for controlling the flow of fluid through a MHD-driven fluidic network comprising a plurality of connected and individually controlled conduits each having a pair of opposing walls and at least one pair of electrodes disposed along the opposing walls, comprising the step of implementing an activation sequence of electrical

currents or potentials across the electrode pairs by means of at least one electrode controller governed by an algorithm and in operable engagement with the electrode pairs.

Yet another aspect of the present invention is a method for generating chaotic advection within a conduit or chamber of a microfluidic network having at least two electrodes disposed therein comprising the step of applying an electrical current or potential across the electrodes to generate at least two different alternating flow patterns and induce chaotic advection. In one embodiment, the current or potential is alternately applied between a stirring electrode and at least two different electrodes disposed along the internal walls of the conduit. The two electrodes may be disposed on the same wall, on adjacent walls or on opposing walls of the conduit. In an alternative embodiment, the current or potential is alternately applied between one electrode disposed along an internal wall of the conduit and at least two stirring electrodes positioned within the conduit and away from the internal wall. In another embodiment, the polarity of the electric field between the one or more stirring electrodes and the one or more electrodes disposed along the internal walls is repeatedly reversed. The Lorentz forces generated by the configuration of electrodes and applied currents or potentials within the conduit result in secondary flows, and in particular flows characterized by chaotic advection, which are effective in mixing laminar fluids such as fluids present within a microfluidic network.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary and the following detailed description of exemplary embodiments of the invention are better understood when read in conjunction with the accompanying drawings.

Figure 1a is a top view of a conduit of a MHD microfluidic network

Figure 1b is a cross-section view of the conduit shown in Fig. 1a.

Figure 2a is a cross-section view of an arrangement of electrodes within a conduit in accordance with one embodiment of the invention.

Figure 2b is a cross-section view of an arrangement of electrodes within a conduit in accordance with another embodiment of the invention.

Figure 3a is a top view of a microfluidic network lacking physical walls with its conduit boundaries defined by the layout of electrodes.

Figure 3b is a cross-section view of the microfluidic network shown in Fig. 3a.

Figure 4 is a schematic representation of a fluidic network with individually-controlled branches numbered 1 through 6 and nodes labeled a through e. An inlet port is located at node a, and exit ports are located at nodes c and e.

Figure 5 is a schematic representation of a MHD fluidic network with individually-controlled branches numbered 1 through 9 and reagent reservoirs denoted R_1 and R_2.

Figure 6 is an exploded view of the fluidic network shown schematically in Figure 5 depicting the layers used in the construction of the device.

Figure 7 is a schematic representation of a MHD network that can be used for combinatorial interactions.

Figure 8 depicts a time-series of images of the predicted and observed stirring of a drop of material inserted in the conduit when two internal electrodes are alternately engaged.

Figure 9 is a cross-section view of a cylindrical MHD stirrer having two internal, eccentrically-located electrodes A and B and a peripheral electrode C.

Figs. 10a through 10i depict Poincaré sections (stroboscopic images) of the trajectories of passive tracers (Figs. 10a through 10c), actual passive tracers' trajectories (Figs. 10d through 10f), and flow visualizations (Figs. 10g through 10i) of traces of a drop of dye inserted in the cavity as functions of the alternations period (T).

Figure 11 is a schematic representation of another embodiment of a MHD stirrer in which the electrodes are transverse to the conduit's walls and they are labeled A_i (i=....-2, -1, 0, 1, 2,...).

Figure 12 depicts flow visualization experiments using a stirrer configured as depicted in Fig. 11.

Figure 13 illustrates the device depicted in Fig. 11 inducing chaotic advection by the deformation of a straight line of dye initially injected midway between the conduit's walls through the application of alternating potential differences between the evenly and oddly numbered electrodes. The figures in the left and right columns correspond, respectively, to theoretical predictions and experimental observations.

Figure 14 depicts schematically a continuous flow thermal cycler that can be used for PCR. A1, A2, B1, B2, C1, C2, D1, and D2 are electrodes. The different shades of gray scale denote zones maintained at different temperatures.

DETAILED DESCRIPTION OF THE INVENTION

Controlled-flow MHD fluidic networks, thermal cyclers, and chaotic advection stirrers for use in microfluidic devices for processing and analyzing biological and chemical samples such as laboratories on chips and micro-total analysis systems are described.

The controlled-flow MHD fluidic network comprises a plurality of connected and individually controlled conduits for the transmission of fluid each conduit having a pair of opposing walls, at least one pair of electrodes disposed along the opposing walls of the conduits, and at least one electrode controller in operational engagement with the electrodes for implementing an activation sequence of currents or potentials across the electrode pairs. In preferred form, the network further comprises an algorithm for determining the activation sequence. Movement of fluid through the network is accomplished in accordance with principles of magnetohydrodynamics which utilize the interaction of approximately perpendicularly oriented electric and magnetic fields to generate Lorentz forces within the network. The pattern of Lorenz forces moving fluid through the network is governed by the activation sequence which defines the particular magnitude, polarity and timing of current or potential to be applied across individual electrode pairs. The activation sequence itself is determined by an algorithm which may produce a predetermined activation sequence or a sequence which uses information about the state of the fluidic network or of the fluid circulating within the network during operation of the network. In this manner, fluid within the network that is at least slightly conductive may be directed with precision and control through the network along any desired path and without the need for mechanical valves or pumps.

The basic building block of the controlled-flow MHD fluidic network is the conduit. Described generally with reference to Figs. 1a and 1b, the individual conduit of the fluidic network has length L, width W, and height h. The conduit may be capped, as depicted in Figs. 1a, 1b, 2a and 2b, or open from above as shown in Fig. 6. Moreover, conduits comprising a network of conduits may have the same or different shapes, lengths and sizes provided that the

conduits are capable of bearing electrodes positioned suitably for the generation of Lorentz forces upon the application of a current or potential within a magnetic field. Suitable configurations include, for example, rectangular, as shown in Figs. 2a and 2b. Alternatively, the conduits may comprise straight, curved or slanted walls that in cross-section are square, trapezoidal, circular, oval, or any other such suitable shape or combination of shapes.

The network of conduits may be simple or complex comprising any combination of curved or straight conduits with few or many interconnections arrayed in either two or three dimensions. A network comprising solely of straight conduits is shown in Fig. 7, and Fig. 5 depicts an example of a network comprising a combination of straight and curved conduits. Further, Figs. 4, 5, and 7 provide schematic depictions of embodiments of relatively simple, two-dimensional networks. In Figs. 4 and 5, the individual conduits, similar in structure to the conduit depicted in Figs. 1a and 1b, are denoted with numbers. The network can be connected to external supplies and drains denoted as a, c, and e in Fig. 4. Alternatively, fluid can circulate inside the network without external links as shown in Fig. 5.

The MHD-controlled networks can be fabricated from a variety of substrate materials including, for example, silicon, monomers, prepolymers, polymers, elastomers, glass, plastics, metals (in combination with dielectric materials) and ceramic materials such as, for example, low temperature, co-fired ceramic tapes. Ceramic tapes are a convenient substrate material as they are dielectric and amenable to layered manufacturing techniques. Individual tapes may be machined and electrodes, conductors, and resistors may be printed or otherwise applied on the tapes with metallic pastes or inks in their green (pre-fired) state when the tapes are soft and pliable. A plurality of individually processed tapes can then be stacked, aligned, laminated, and

co-fired to form a monolithic device that integrates hydraulic conduits and conductive paths arrayed in a two or three dimensions. Such manufacturing techniques also provide a means for inexpensive and rapid prototyping. Ceramic tapes may further include magnetic materials such as magnetic particles thus integrating the magnetic field source into the substrate and eliminating the need for the use of external magnets to generate Lorentz forces within the network.

In the pre-fired (green) state, ceramic tapes may comprise oxide particles such as alumina and/or silica, glass frit, and an organic binder that can be made from photo-resist. The tapes are available in a variety of thicknesses, typically from about 100 μm±7% to a few hustred microns, although thinner tapes of about 40 μm can be casted. The tapes in their green state are soft and pliable, and can be readily machined by a variety of known techniques including laser, milling, and photolithography when the binder is photo-resist. Conductive paths such as metallic circuits may be either printed, processed photolithographically, or otherwise applied to the tapes to form electrical circuits and components such as electrodes, resistors, conductors, and thermistors. Conduit sizes may be any size suitable for use in MHD-driven fluidic networks, and in one embodiment may range from about 10 μm to several millimeters. Individual tapes may be stacked, aligned, laminated, and co-fired to form sintered, monolithic structures having complex, either two- or three-dimensional networks of fluidic conduits, electronic circuits, and electrodes. Glass or other materials can be attached to or incorporated in the tapes to facilitate optical paths. Further, the tapes may include a magnetic material such as magnetic particles and/or a single or multiple layers of coils may be embedded in the tapes to generate a magnetic field.

The conduits of the fluidic network are provided with at least one pair of electrodes (denoted in Figs. 1a and 1b as C_U and C_D of length L_e) positioned on opposing internal surfaces

of the conduit. These electrodes, referred to as driving electrodes, define the region along the conduit in which an electric current or potential is applied. The driving electrodes may be positioned on the internal surfaces of the conduits in a variety of ways all of which are considered within the scope of the invention. Two such electrode configurations are depicted in Figs. 2a and 2b. Fig. 2a depicts an arrangement of electrodes comprising four individual electrodes positioned along the corners of a conduit as shown in cross-section. Fig 2b depicts an arrangement of electrodes comprising a pair of individual electrodes each covering the entire area of opposing sidewalls of a conduit. The arrangement of electrodes as shown in Fig. 2b is a configuration that can provide a nearly uniform current density in a fluid within the conduit. Electrodes may also be used to control the shape of the velocity profile and, depending on the specific application, arrangements other than those shown in Figs. 2a and 2b may be preferable.

It is further aspect of the invention that not every individual conduit within the network need be provided with driving electrodes provided that conduits that are not so equipped are in communication with at least one conduit that is so equipped. In this manner, the propulsion of fluid through the conduit having driving electrodes is capable of driving fluid through the conduit lacking such electrodes. In preferred form, the driving electrodes terminate some distance away from the ends of the conduit so as to minimize current leakage (cross-talk) between or among adjacent conduits comprising the network.

The driving electrodes themselves may be used to form virtual conduits, that is, conduits which lack physical walls for the containment of the fluid. Flow of fluid through a network comprising virtual conduits is spatially defined by the configuration of the electrodes on the substrate and controlled by the current or voltage applied across electrode pairs. In such

networks, the electrodes may either protrude from, be flush with, and/or terminate beneath the surface of the substrate. Figs. 3a and 3b depict an example of a toroidal virtual conduit and a straight virtual conduit in which the "walls" of the conduits are the electrodes themselves. Figs. 3a and 3b correspond, respectively, to a top view and a view in cross-section of the virtual conduits. Complicated patterns of electrodes may be readily manufactured using a variety of printing and lithographic techniques for applying the electrodes to the substrate.

Each pair of driving electrodes is in operable engagement with an electrode controller that acts to control the magnitude, polarity and timing of the current or potential applied across pairs of driving electrodes. The network may comprise a single electrode controller in operable engagement with each of the driving electrodes of the network. Alternatively, the network may comprise a plurality of electrode controllers each of which controls one or more driving electrodes of the network. In one embodiment, each pair of driving electrodes is controlled by a separate electrode controller. By controlling the current and/or potential applied across each electrode pair, the one or more electrode controllers regulate in a precise pattern and with precise timing the generation of Lorentz forces that propel the fluid through the conduits of the network. An implementation of an exemplary electrode controller and algorithm are described in greater detail in Bau, H., H., Zhu, J., Qian, S., and Xiang, 2003, Y., *A Magneto-Hydrodynamically Controlled Fluidic Network*, Sensors and Actuators B: Chemical, 88, 205-216, which is incorporated herein in its entirety.

The one or more electrode controllers of the network are governed by an activation sequence that coordinates and controls the flow and combination of fluids within the network. In one embodiment, the activation sequence is determined in accordance with an algorithm which

computes and defines the magnitudes, polarities and timing of currents or potential differences applied across the various driving electrode pairs of the network that are necessary to achieve the desired control of flow paths and flow rates throughout the network. The algorithm can be in any form that is capable of determining the specific current or potential across various electrode pairs throughout the network associated with generating a precise pattern of Lorentz forces for propelling the fluid in a controlled manner along any desired path through the network including, for example, an equation, a series of equations, a series of iterative steps, or software. In one embodiment, the user specifies the desired flow path and the flow rates associated with the various conduits. The algorithm then computes the magnitudes, polarities and timing of currents or the voltages that are needed to implement the desired conditions. The algorithm may also compute the magnitudes, polarities and timing of currents or voltages while minimizing an objective function such as, for example, the total power dissipation of the device. The sequence of specific magnitudes, polarities and timing of currents or voltages across particular electrode pairs comprises the activation sequence that is used by the electrode controllers to generate the Lorentz forces necessary to propel fluid in the network along the desired flow path.

Preferably, the algorithm is in the form of a software program capable of calculating specific magnetic and/or electric field strengths associated with flow rates within conduits of a known size. As a software program, the algorithm may be resident on the one or more electrode controllers or located remote from the controllers provided the activation sequence generated by the algorithm is capable of communication with and implementation by the one or more electrode controllers. The algorithm may determine the magnitude, polarity and timing of current or potential in a predetermined mode or in a mode that uses feedback generated by the

operation of the network in determining the activation sequence. In embodiments in which the activation sequence is determined at least partially with the use of feedback, the network further comprises a sensor assembly capable of continuously or periodically collecting information about the state of the network or the fluid circulating within it during operation and inputting this information into the algorithm. MHD-driven fluidic networks in which movement through the network can be controlled by an activation sequence generated by an algorithm are suitable for a variety of applications including point-of-care medical diagnosis; laboratory diagnosis; drug discovery; air, food, and water quality monitoring; and detection of pathogens and chemical agents associated with biological and chemical warfare agents.

In accordance with MHD principles, the orientation of the magnetic field need not be vertical with respect to a conduit oriented in a horizontal plane. For example, if a pair of driving electrodes were positioned on the top and bottom walls of the conduit oriented in a horizontal plane, MHD principles would require the magnetic field to be oriented also horizontally but transverse to the direction of flow of the conduit. Thus, the controlled-flow MHD fluidic network of the present invention may accommodate any combination of electrical and magnetic fields that are approximately perpendicular to each other and in any orientation with respect to the conduit provided both fields are approximately perpendicular with respect to the axis of flow through the conduit. In one embodiment, the conduits comprising the network are arranged in a planar configuration and the magnetic field is oriented approximately perpendicular to the plane in which the conduits are arrayed.

As shown in Figs. 1a and 1b, a three-dimensional Cartesian coordinate system can be represented with respect to an exemplary conduit of the network by axes x_1, x_2, and x_3. The

vertical arrow denoted with the letter (B) indicates the orientation of the magnetic field. In operation, the entire device is subjected to a magnetic field of a specific intensity. The magnetic field may be generated by a permanent magnet or an electromagnet. Alternatively, the entire network may be fabricated with the inclusion of a magnetic material, thereby eliminating the need for an external magnetic field source. The most suitable source for the magnetic field will depend on a variety of factors including the particular application for which the network is intended. In other embodiments, synchronized, alternating electric and magnetic fields may also be used. In such embodiments, the fields may be synchronized such that the resulting Lorentz forces remains essentially steady.

Fluid is transmitted from one region of the MHD network to another by currents I_i or potential differences V_i applied across the driving electrode pairs within the conduits of the network. The potential difference V_i in a given conduit (i) induces an electric current of density $J_i \sim s_i V_i / W_i$ where W_i is the conduit's width and s_i is the specific electric conductivity of the fluid. This current, in turn, interacts with the magnetic field to produce a Lorentz body force of density ($J_i B$) directed along the axis of the conduit. The magnitude of the force and its direction may readily be controlled by respectively controlling the magnitude and polarity of either the potential difference V_i or the current I_i. Since the relationship between the flow rate and the current is linear over the domain of interest, the electric current typically will be the preferred control variable. To a first approximation, the flow rate (Q_i) in the conduit is given as a function of the potential difference V_i (or the current I_i), and the pressure drop across the length of the branch (DP_i) by the constitutive relationships of the type: $Q_i = H_i DP_i + M_i^V V_i$ or $Q_i = H_i DP_i + M_i^I I_i$ where H_i and M_i are, respectively, the hydraulic and MHD conductivities. Preferably, the

conduits comprising the network are sufficiently long so that fringe effects can be neglected, and the current flow is essentially one-dimensional.

An exemplary MHD-controlled fluidic network is shown in Fig. 4. The individual conduits comprising the network are denoted by the numbers 1 through 6 and the nodes are denoted with the letters a through e. Nodes a, c, and e communicate beyond the network or with reagent reservoirs and serve as sinks and sources. In an alternative embodiment, the network is not provided with any sinks or sources. Conduits which do not contain driving electrodes have hydro-magnetic conductivity set to zero. For the network depicted in Fig. 4, six equations relate the flow rate in a conduit to the pressure drop along that conduit's length and the potential difference across the electrodes. Additionally, mass continuity (Kirchhoff's law) requires that all the flow rates arriving at each node sum up to zero. When the potential differences across all the conduits and the pressures at the sources and sinks are given, these equations can be solved to obtain the flow rates in all the conduits.

An embodiment of the network as shown in Fig. 5 is manufactured with low temperature, co-fired ceramic tapes. The device has planar architecture, that is, all the conduits of the network are arrayed in a single layer. While the single conduit layer shown in Fig. 5 consists of a plurality of tapes, an individual layer may be formed from a single tape or from a series of tapes depending on the thickness of the tapes and the desired layer thickness. In an alternative embodiment, conduits may be fabricated in multiple layers and interconnected through one or more vertical wells to form a network comprising a three-dimensional array of conduits.

In one embodiment, a planar, MHD-controlled fluidic network is fabricated with LTCC 951AX co-fired ceramic tapes supplied by DuPont that have a nominal (pre-fired) thickness of

~250 μm. Fig. 6 provides an exploded view of the elements of the network as shown schematically in Fig. 5. The fabrication process consists of blanking rectangular segments of tapes to a desired size. A few layers of tapes are laminated to form a part. The various parts are machined individually using a numerically-controlled milling machine. Subsequently, electrodes and conductor paths are printed on the various parts.

In one embodiment as shown in Fig. 6, layer A is the top layer that contains the flow conduits and includes 1.1 mm wide x 1.7 mm deep flow conduits and soldering pads using DuPont 6134 solderable conductors. The soldering pads are connected through vertical vias filled with DuPont 6141 via fill paste to the various electrodes. While relatively large conduits are fabricated in this embodiment to facilitate easy flow visualization, similar networks may be fabricated having much smaller dimensions. Layer B comprises the bottom wall of the conduits and contains the electrodes and some of the electrical leads connecting to the electrodes. A more detailed layout of the electrodes shown in layer B is provided in insert E. About 20 μm thick x 2 mm wide electrodes made from DuPont 5734 gold paste are printed on the surface of layer B. The gold electrodes are aligned with the edges of the conduits such that when layers A and B are attached, about 0.1mm of the widths of the electrodes along each side of the conduits' vertical walls are exposed to the conduits. Each conduit is provided with a pair of driving electrodes. A gap separates the driving electrodes in adjacent conduits. Silver conductors made from DuPont 6145 conductor paste are printed on both layers B and C to facilitate the connection of each electrode to the soldering pads located on the surface of layer A. All the leads are connected through vertical vias to terminals located on the surface of layer A. Layer D, the bottom layer, contains additional leads. Subsequent to machining and printing, the individual parts are

stacked, aligned, laminated, and co-fired to form a sintered, monolithic block. The device may capped with a cover plate or left uncapped to facilitate easy access to the channels and to enable dye injection for flow visualization.

In one embodiment, the electrodes of the network may be controlled by an electrode controller comprising computer-controlled relay actuators and a D/I card. The relays are programmed to switch on and off in such a way that any one or combination of electrode pairs in the network can be active at any given time and for any given interval. Additionally, the relays allow for the switching of the polarity of any given pair of electrodes and the supply of power either in controlled-voltage or controlled-current modes.

In operation, the conduits are filled with a fluid that is at least slightly conductive such as, for example, saline solution. While 0.1M and 0.3M solutions are suitable, MHD-driven networks can operate with ion concentrations as low as about 50mM. The device is placed on top of a neodymium (NdFeB) permanent magnet of approximate intensity B=0.4T (Edmund Scientific). Dye (Cole Parmer Instrument Co.) is injected at various locations to achieve flow visualization.

The fluidic network may be analyzed using linear graph theory methodology, and the potentials V_i or currents I_i may be determined so as to direct the fluid to follow any desired path. In one set of experiments utilizing a network configured as shown in Fig. 5, a trace of dye was inserted into the fluid at conduit 1. The electrodes of all the conduits were activated, and the network was programmed to pump the fluid around the large circuit (conduits 1, 2/3, 4, 5/ 6, and 7) with the flow divided between conduits 2 and 3 and between conduits 5 and 6. By appropriate choice of current or potential differences, the flow can be split between conduits 2 and 3, and

between conduits 5 and 6 in any desired proportion. When the dye entered the torus comprising conduits 2 and 3, the electrodes within all the conduits but 2 and 3 were switched off. The polarity of conduit 3's electrodes was reversed, and the fluid was forced to circulate around the torus. In one embodiment, the torus can be positioned across a temperature gradient having two or more different temperature zones such as may be needed for DNA amplification reactions. Subsequently, the polarity of electrodes in conduit 3 was reset to its original setting, all the other electrodes were turned on, and the dye was pumped out of the torus into conduit 4. The dye then split between conduits 5 and 6 and recombined in conduit 7. By appropriate choice of current or potential differences, the flow can be made to circulate around the loop consisting of conduits 6 and 7. In an alternative network, liquids are pumped from the wells at the end of conduits 8 and 9, the fluids are mixed in conduit 1 equipped with stirring electrodes as described herein, and the electrodes are programmed to pump the liquid into the torus defined by conduits 2 and 3.

Figure 7 depicts schematically a more complicated MHD network consisting of a plurality of wells R and conduits 10 through which reagents, analytes, or chemicals may be pumped along any desired path and stirred, causing various interactions and/or reactions. Each of the conduits shown in Fig. 7 has a structure similar to the conduit depicted in Fig. 1. Analytes and reagents may be pumped from any of the wells, brought together, and mixed to interact and/or react with reagents pumped from other wells. The network may also facilitate combinatorial screening in which many processes are carried out in parallel. Moreover, reaction and interaction products may be used in subsequent reactions or interactions in either pre-determined or feedback modes. The embodiment depicted in Fig. 7 can readily be expanded to a three-dimensional network allowing a much larger number of connections. These examples

illustrate that MHD-controlled networks provide an easy, effective and inexpensive way of circulating fluids through microfluidic laboratory on a chip conduits.

MHD-controlled networks can operate with a wide variety of electrolyte and buffer solutions such as, for example, solutions containing NaCl, KCl, NH_4Cl, $CuSO_4$, $FeCl_2/FeCl_3$, NaH_2PO_2, and Hydroquinone among many others. The performance of the device, however, may be affected by the particular solution and electrode materials that are used. For example, the use of NaCl solutions may lead to bubble production at relatively high current densities and electrode corrosion. To the extent MHD-driven devices are used as disposable devices, electrode corrosion may not be an issue of significance. Moreover, the MHD-driven devices, depending on the application, can operate either open or capped. With open conduits, bubble formation may not present a problem. In closed conduits, however, bubble generation must be addressed and preferably limited. In one embodiment, the use of redox species such as $FeCl_2$ / $FeCl_3$ solution with platinum electrodes may sustain higher current densities than a NaCl solution without bubble formation and without electrode corrosion. Ultimately, though, the choice of the electrolyte or buffer is dictated by, among other things, the compatibility of the electrolyte or buffer with the specific processes to be performed in the system. In addition to the MHD forces, the fluid within the network may also be subject to a pressure gradient that is either flow assisting or flow adverse.

Another aspect of the present invention is an MHD stirrer. Chemical reactions and biological interactions in a microfluidic device often involve mixing or stirring fluids in order to bring various molecules together. Mixing by diffusion alone in a microfluidic device is often not efficient. The diffusion time of macromolecules may be prohibitively large even when the

lengths are measured in hundreds of microns. Moreover, since flows are often laminar and corresponding Reynolds numbers in microdevices are usually very small, one is also denied the benefits of turbulence as an efficient mixer.

In one embodiment, the MHD stirrer of the present invention comprises a conduit or chamber having at least two electrodes disposed therein such that the application of a current or potential across the electrodes within a magnetic field generates secondary flows such as flows characterized by chaotic advection. In embodiments in which two electrodes are used to induce chaotic advection, at least one electrode must be movable so that the current or potential may be applied alternately across electrode pairs in at least two positions. In embodiments in which at least three electrodes are used, the electrodes may be movable or fixed and disposed along and/or away from the internal walls of the conduit or chamber. In either embodiment, a current or potential is alternately applied across electrodes occupying at least three positions to induce at least two alternating flow patterns which generates chaotic advection.

In one embodiment, the MHD stirrer of the present invention comprises a conduit having at least one electrode disposed along the wall of the conduit, and at least two electrodes positioned within the conduit and away from the wall. In another embodiment, the MHD stirrer comprises at least two electrodes disposed along at least one wall, and at least one electrode positioned within the conduit and away from the wall. In a further embodiment, the stirrer has at least two electrodes aligned along at least one wall, and at least one electrode disposed along another wall. In yet another embodiment as shown in Figs. 1a and 1b, the MHD stirrer comprises a pair of electrodes disposed along the opposing walls, and at least two electrodes positioned within the conduit and away from the opposing walls. In this embodiment, the

electrodes positioned within the conduit and away from the opposing walls may be aligned as shown in Fig. 1a along the centerline of the conduit's bottom. In still another embodiment, the MHD stirrer comprises a cylindrical chamber with an electrode disposed around its internal periphery and at least two electrodes positioned eccentrically inside the chamber. The placement of the one or more electrodes permits the generation of complex secondary flows including flows characterized by chaotic advection that is beneficial for mixing or stirring within a fluidic conduit or chamber. The conduits as described in all of these embodiments may comprise a conduit of the MHD-driven fluidic network or thermal cycler of the present invention.

MHD stirrers that generate chaotic advection may operate either by varying the current or potential applied across electrode pairs between zero and a prescribed value (either positive or negative) or by repeatedly reversing the polarity of each electrode by varying the current or potential between negative and positive values. Depending on the particular electrolyte used, reversal of polarity may be advantageous in certain cases since by reducing electrode corrosion and bubble accumulation on electrode surfaces. Furthermore, in applications in which analyte migration in the electric field is a problem, reversing polarity is likely to reduce or eliminate such migration.

In the embodiment shown in Figs. 1a and 1b, a conduit of a controlled, MHD-driven microfluidic network is provided with a pair of electrodes disposed on opposing walls of the conduit and a series of electrodes denoted A_i disposed along the centerline of the conduit and away from the opposing walls. Electrodes A_i (where $i = 1, 2, 3,\ldots$) are referred to as stirring electrodes. This particular implementation of the stirrer is described in greater detail in Qian, S., Zhu, J., and Bau, H. H., 2002, *A Stirrer for Magneto-Hydrodynamically Controlled Micro*

Fluidic Networks, Physics of Fluids, 14 (10): 3584-3592 which is incorporated herein in its entirety.

In order to operate a MHD conduit as a stirrer, the electrodes intended for use in creating secondary flows are in operable engagement with at least one electrode controller such as, for example, a computer-controlled relay actuator. In one embodiment, relay-actuators combine both driving electrodes C_U and C_D into a single electrode C. When a potential difference is applied across the electrode pair C-A_i, circulatory motion of the fluid within the conduit is generated, with the fluid circulating around electrode A_i. When the electrode pair C-A_1 is activated for a time interval T_1, electrode pair C-A_2 for another time interval T_2, then electrode pair C-A_1 once again, and so on in a periodic fashion, chaotic advection is generated. As the magnitude of the period ($T=T_1+T_2$) increases, the chaotic region increases in size and complexity. In some circumstances, it may be advantageous to alternate the electrode potentials in a non-periodic fashion.

In demonstrating this effect, flow visualization experiments of the stretching and deformations of a dye blob were performed. Fig. 8 depicts computational and experimental results when a blob of dye was inserted into the conduit and the evolution of the dye was tracked over time. Both in experiment and theory, a rapid spread of the dye was observed indicating efficient stirring. By engaging a larger number of electrode pairs C-A_i, one can further extend the fraction of the conduit that participates in the mixing process. As electrodes may be readily patterned into various shapes, electric fields may be induced in different directions. The interaction of such electric fields with the magnetic field can be used to induce secondary complex flows that may be beneficial for stirring and mixing

Stirring electrodes such as electrodes A_i shown in the embodiment depicted in Figs. 1a and 1b may be located singly or in combination anywhere within a conduit provided they are away from either of the opposing walls. The electrodes need not to be aligned along the conduit's center. Although it is convenient to print the electrodes on the device's floor to avoid intrusion, one can also use other arrangements such as, for example, electrodes in the form of pins that protrude into the conduit.

The MHD stirrer may comprise either an open or a closed cavity of any suitable shape. With reference to Fig. 9, an embodiment of the stirrer is described comprising a circular cavity with an electrode C deposited along its periphery. Two additional electrodes, A and B, are deposited on the cavity's bottom. The cavity is filled with a conducting liquid such as, for example, a saline solution, and it is positioned in a uniform magnetic field oriented parallel to the cavity's axis. When a potential difference is imposed across the two electrodes A and C, an electric current flows between the two electrodes and the interaction between this current and the magnetic field results in Lorentz forces that induce, for example, counterclockwise flow circulation in the cavity centered next to the location of electrode A. Subsequently, when the potential difference is switched from electrode pair A/C to electrode pair B/C, depending on the polarity of the electrodes, either a counterclockwise or a clockwise circulatory pattern may be induced centered next to the location of electrode B. The device is operated by alternately engaging electrode pairs A/C and B/C with a period T. In some circumstances, it may be advantageous to alternate the electrode potentials in a non-periodic fashion.

Figs. 10a through 10i depict Poincaré sections (stroboscopic images) of the trajectories of passive tracers (Figs. 10a through 10c), actual passive tracers' trajectories (Figs. 10d through

10f), and flow visualizations (Figs. 10g through 10i) of traces of a drop of dye inserted in the cavity as functions of the normalized (dimensionless) alternations period (T). The columns of figures (10a, 10d and 10g), (10b, 10e and 10h) and (10c, 10f and 10i) correspond, respectively, to T = 2, 6 and 8. When T = 2, the motion depicted in the Poincaré section shown in 10a is mostly regular and consists of two sets of closed orbits, one set encircling one electrode and the other set encircling the other electrode. Fig. 10d depicts the actual motion of the tracer which shows the presence of jitters resulting from the tracer being trapped (at different times) by the flow fields induced by the two electrode pairs. The presence of two families of periodic orbits is well supported by the flow visualization experiments as shown in Fig. 10g.

As the period T increases, chaotic islands become visible. Fig. 10e illustrates that as the period increases, so does the magnitude of the jitters. The presence of the global structure consisting of two counter-rotating circulations is visible in Fig. 10h. When T = 8, Fig. 10c depicts the trajectory of a single tracer. The irregular chaotic region appears to have spread to cover almost the entire cavity. Similar to Fig. 10c, the flow visualization experiments depicted in Fig. 10i illustrate the presence of counter-rotating eddies through the existence of an unmixed zone at the ends of the diagonal that is perpendicular to the line connecting the two electrodes. The operation of the device is described in greater detail in Yi , M., Qian, S., and Bau, H. H. , *A Magneto-hydrodynamic (MHD) Chaotic Stirrer*, J. Fluid Mechanics, 468, 153-177 (2002) which is incorporated herein in its entirety.

Fig. 11 depicts a schematic representation of another embodiment of a MHD stirrer. As shown in Fig. 11, the stirring electrodes are aligned perpendicular to the conduit's walls. By subjecting these electrodes to varying potential differences in the presence of a magnetic field,

forces are generated that drive fluid flow in various directions in "virtual" conduits whose geometry is dictated by the positioning of the electrodes. An implementation of the stirrer is described in greater detail in Bau, H. H., Zhong, J., and Yi, M., 2001, *A Minute Magneto Hydro Dynamic (MHD) Mixer*, Sensors and Actuators B, 79/2-3, 205-213; and Xiang, Y. and Bau, H. H., 2003, *Complex Magneto Hydrodynamic, Low Reynolds Number Flows*, Physical Review Letters E, 68, 016312-1 – 016312-11, which are incorporated herein in their entirety.

Fig. 12 depicts the deformation of an initially straight dye line resulting from the application of Lorentz forces by means of a MHD stirrer of the present invention. A thin trace of dye (Water Soluble Fluorescent Liquid Dye, Model 298-16-Red, Cole Palmer Instrument Company, Niles, Illinois, USA) is applied by means of a syringe across the cavity and then a potential difference is applied across adjacent electrodes. As a result of the application of the potential difference, fluid flow is induced in the cavity. The motion consists of rotating cells with the fluid moving up in one interval between two electrodes and down in the adjacent interval. Frame A in Fig. 12 depicts the line of dye initially inserted into the device. Depending on the polarity of the electrodes, the dye either moves upwards or downwards as shown in frame B of Fig. 12. After a few seconds, the polarity of the electrodes is reversed. Since diffusion is relatively slow and the flow is at a relatively low Reynolds number, the dye retracts its steps in almost a reverse fashion as shown in frame C of Fig. 12 and then starts deforming in the opposite direction as shown in frame D of Fig. 12. When the process is allowed to continue for some time, the dye traces the convective cells as shown in frame E of Fig. 12 in good qualitative agreement with theoretical predictions.

The electrodes may be patterned in many different ways to induce various flow patterns. The embodiments described above are just a few examples of numerous possible variants of MHD stirrers.

Fig. 13 compares theoretical predictions shown in the left column and experimental results shown in the right column obtained in another implementation. Figure 13 depicts the deformation of an initially straight line of dye under various operating conditions. The top row depicts the flow structure when only the odd-numbered electrodes are active. By alternating the potential difference across non-adjacent pairs of electrodes, it is possible to induce chaotic motion in the cavity. For example, electrodes A_{-2}, A_0, and A_2 may be engaged for the time interval T_1, and then electrodes A_{-1} and A_1 for the time interval T_2. By repeating this mode of operation, fairly complicated flows are generated and effective stirring is provided. The results of this mode of operation are depicted in the bottom row of Fig. 13.

Another aspect of the invention is a controlled, MHD-driven thermal cycler comprising the fluidic network of the present invention positioned at least partially within a temperature gradient. Magnetohydrodynamics provides the means to circulate fluids continuously in a closed loop. Different parts of the loop may be maintained at different temperatures, enabling the cycling of the liquid to subject the liquid to different temperature zones.

Fig. 14 depicts one embodiment of the thermal cycler of the present invention. The cycler comprises a closed conduit loop with electrodes aligned along opposing walls. Electrodes A1 and B1 are aligned along the inner wall of the loop, and electrodes A2 and B2 are aligned along the outer wall of the loop. An entry port with electrodes C1 and C2 aligned along its opposing walls leads into the loop and an exit port with electrodes D1 and D2 aligned along its

opposing walls leads out of the loop. While the device shown in Fig. 14 has one inlet and a separate exit port, the cycler may be equipped with a single port or larger number of inlet and exit ports. In order to utilize the conduit loop depicted in Fig. 14 as a thermal cycler, different parts of the loop are maintained at different temperatures. The various temperature zones may be maintained, for example, with the use of electrical resistors or thermoelectric units (not shown). Fig. 14 depicts three thermal zones. It is contemplated as within the scope of the invention that a larger or a fewer number of thermal zones may be used as is suitable with reference to the particular application.

At the beginning of operation, an electrical potential is applied to the electrodes such that the material is drawn into the loop. The polarities of either electrode pair A1 and A2 or electrode pair B1 and B2 are then reversed so that the material within the conduit loop is forced to circulate continuously around the loop. The particular choice of polarity will determine whether the motion is in the counterclockwise or clockwise direction. If necessary, the polarities and the magnitudes of the potentials applied to electrodes C1, C2, D1, and D2 may be adjusted so as to prevent the material within the conduit from leaving the loop. Also, if necessary, the direction of the flow in the thermal cycler may be periodically changed to minimize analyte migration in the electric field. As the material within the conduit cycles around the loop, it is exposed to different temperatures. In certain embodiments, this cycling between or among different temperature zones facilitates biological interactions such as, for example, those needed for PCR.

After the material within the conduit has completed the desired number of cycles around the loop, electrical potentials are supplied to the various electrodes so as to pump the reaction products out of the loop. The reaction products may be pumped either through the exit port

defined by electrodes D1′ and D2, back through the inlet port defined by electrodes C1 and C2, or split among any number of exit ports (not shown in the figure) so as to transport parts of the sample to different subsequent analysis paths. The embodiment of the MHD thermal cycler depicted in Fig. 14 may be readily integrated into a magneto-hydrodynamic network such as the one depicted in Fig. 6, integrated into a network in which fluids are propelled by other means than MHD, or used as a stand-alone device. In all three implementations, MHD stirrers of the present invention may be integrated into the MHD thermal cycler of the present invention to enhance efficiency.

One application of the MHD thermal cycler is for PCR. The MHD thermal cycler has the advantage over other continuous flow devices in that the number of cycles may be readily adjusted in a predetermined mode according to the characteristics of the analyte to be amplified or in a feedback mode with the use of a sensor capable of detecting the amplification rate. Since it is not necessary to cycle the substrate temperature as is done in conventional PCRs, the MHD thermal cycler is capable of facilitating rapid amplification of DNA.